Survival of Cheese Starter Cultures During High Pressure Carbon Dioxide Processing of Milk

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ABSTRACT

The potential of a new process that separates milk into casein curd and whey within minutes was studied to determine if cheese starter cultures could survive the process, producing curds suitable for cheesemaking in significantly less time than traditional methods. Milk, containing 0, 1, or 3.25% fat and inoculated with *Lactobacillus delbrueckii* ssp. *bulgaricus (L. bulgaricus)*, *Lactococcus lactis* ssp. *lactis (L. lactis)*, or *Streptococcus thermophilus (S. thermophilus)*; was processed for five min at 38°C by sparging CO_2 through the milk to obtain a pressure of 5.52 MPa. Survival and acid producing capabilities of cultures were evaluated in the resulting curds. Before processing, inoculated milk pH was 6.5 to 6.6 and contained 6.7 to 7.4 log colony forming units (CFU)/mL; after processing, curd pH was 5.61 to 5.65 and contained 5.5 to 7.4 log CFU/g and whey pH was 6.23 to 6.24 and contained 5.0 to 6.7 log CFU/mL. After accelerated aging for 2 days at 30 or 37°C, curd pH was 4.53 and 5.22. The results show that processing inoculated milk with CO_2 under pressure produced curds with sufficient viable cheese starter microorganisms to continue lowering the curd pH. With some draining, the curds were ready for the next step in cheesemaking.

(Key words: carbon dioxide, casein, cheese cultures, milk, whey)

INTRODUCTION

With over 7.4 billion pounds of cheese manufactured in the US in 1998 and an expected 8 billion pounds in 2001 (Anon., 1999), increasing consumer demands have challenged dairy researchers to explore new processing methods. Consumers want traditional cheeses as well as cheeses lower in fat and salt and insist on quality nutritional, functionality, and textural cheese characteristics. Coagulation of milk, curd development, and whey drainage have always been critical time-consuming steps in cheese manufacture and major research emphasis has centered on improving the efficiency of these steps.

In recent research on batch processing of milk using CO_2 under high pressure, casein was successfully precipitated, forming curds within minutes (Jordan et al., 1987; Tomasula, 1995). Variations in yield, curd and whey separation, casein solubility, and curd appearance depend on the processing temperature, pressure, and agitation (Tomasula et al., 1995).

Viable cheese starter cultures in the curd lower the curd pH (pH 5.2 is typical) and contribute to the development of the desired cheese flavor and texture. Our major concern

was the ability of these cultures to survive the CO_2 - high pressure treatment. Carbonation of milk, a common practice in the dairy industry to extend the shelf life of milk, significantly reduces psychrotrophic bacteria counts and lowers the pH in fluid milk (Migo et al., 1995; King and Mabbitt, 1982; Raus-Madicdo et al., 1996; Roberts and Torrey, 1988). Cheeses made from carbonated milk have lower psychrotrophic bacterial counts and reduced proteolysis over time (McCarney et al., 1995; Montilla et al., 1995; Uceda et al., 1994). High hydrostatic pressure has severe effects on microorganisms: pressures as low as 0.6 MPa affects cell morphology, pressures below 100 MPa disrupt hydrophobic interactions, and pressures above 100 MPa alter biochemical reactions and the integrity of cell membranes (Hoover et al., 1981).

The goal of this study was to evaluate the potential of this process in preparing curd suitable for cheesemaking. To achieve this, milk (0, 1, or 3.25% fat) was inoculated with one of three common dairy starter cultures and processed with CO_2 at high pressure. The resulting curd was evaluated to determine the effects of the CO_2 treatment on the survival rate and acid producing capabilities of the starter cultures.

MATERIALS & METHODS

Materials

UHT skim milk (Parmalat, Moonachie, NJ) and pasteurized non-homogenized whole and skim milk (Chrome Dairies, Oxford, PA) were purchased locally. Frozen (-60°C) cheese starter cultures (American Type Culture Collection, Bethesda, MD) included: *Lactobacillus delbrueckii* ssp. *bulgaricus (L. bulgaricus)* #11842, *Lactococcus lactis* ssp. *lactis (L. lactis)* #11955, and *Streptococcus thermophilus (S. thermophilus)* #19258, gifts of George A. Somkuti, ERRC, ARS, Wyndmoor, PA. Bacto agar, Bacto peptone, beef extract, Lactobacilli MRS broth (MRS), tryptone, and yeast extract were obtained from Difco Laboratories, Inc., Detroit, MI. Lactose and K_2HPO_4 were purchased from J. T. Baker, Phillipsburg, NJ). Cylinders of liquid CO_2 were obtained from BOC Group, Inc., Murray Hill, NJ. The quaternary sanitizer, Lysol I.C., used to disinfect the CO_2 equipment and the microbiological work areas, was obtained from National Laboratories, Montvale, NJ. All other chemicals were analytical grade.

Preparation of Milk

Fat content of the skim and whole non-homogenized milk was determined using the Babcock assay (AOAC, 1990), and the milk was standardized to obtain 0, 1, and 3.25% fat. Stock cultures of *L. bulgaricus* were maintained in Lactobacillus MRS broth, and *L. lactis* and *S. thermophilus* were maintained in tryptone yeast lactose (TYL) broth, pH 6.5, prepared as described by Hogg and Jago (Hogg and Jago, 1970). Aliquots (0.1 mL) of stock cultures were transferred to separate test tubes containing 20 mL of UHT skim milk and incubated for at least 16h at either 30°C (for *L. lactis*) or 37°C (for *L. bulgaricus* or *S. thermophilus*) One 20-mL culture was used to inoculate 500 mL of milk containing either 0, 1, or 3.25% fat. Inoculated milks were incubated for 3 h at the appropriate temperature with shaking (100 RPM) to insure adequate distribution of the cells without causing air

bubble formation. Control samples were removed at 0 and 3 h for plate counting and pH determination.

Processing

A Parr reactor (Model 4521 316SS, Parr Instrument Co., Moline, IL) with a 1-L capacity was modified as described by Tomasula et al. (1995). The lid of the chamber was fitted with a 3-blade propeller stirrer, a CO_2 sparger made of a modified 2-μ porous metal filter (Supelco, Inc., Belfonte, PA) on a 0.6-cm tube, and a metal filter frame fitted with a piece of silk. The chamber could be tilted and its height adjusted for filling and then raised to seal and secure the lid for pressurization. Before each sample was processed, the sparger and stirrer were removed, and the CO_2 chamber and inlet and outlet lines were rinsed with the sanitizer and then purged with steam for 30 min. The sparger was flamed to remove all particulate material. The sparger, stirrer, and silk filter were sanitized before being reinserted into the chamber.

Approximately 440 mL of inoculated sample were placed in the batch chamber and the chamber sealed. The sample was stirred as it was warmed to 38°C and for the first 4 min of processing to ensure adequate mixing and heat transfer. Chilled CO_2 was slowly sparged into the chamber during the 2 min period until the processing pressure was achieved. Processing conditions of 5.52 MPa and 38°C were held for 5 min before pressure was released.

Microbiological Analysis

After processing, pressure was released by venting CO_2 through the outlet line. Whey was also collected aseptically in a container through the outlet line and placed on ice. Whey was very foamy and required about 10 min to collapse foam and release CO_2 before samples could be taken for plating and pH determination. After the chamber was opened, the casein curd was removed aseptically and placed on cheese cloth suspended within a beaker to allow the excess whey to drain. The beaker was covered with aluminum foil and placed on ice.

Standard pour plate technique (Houghtby et al., 1992) was used to determine colony forming units (CFU)/mL or CFU/g. Samples were serially diluted using 0.1% peptone water. The curd (1-3 g) was prepared for plate count by first making a 1:10 dilution with peptone water and then processed for 1 min at medium speed (Stomacher 400, Teckmar, Cincinnati, OH). The MRS and TYL agars were prepared by adding 1.5% Bacto agar to the broths. Triplicate plates for each dilution were incubated for 48 h at either 30°C (for *L. lactis*) or 37°C (for *L. bulgaricus* and *S. thermophilus*).

Chemical Analysis

After portions of samples were removed for microbiological analysis, the pH was measured for milk, whey, and drained curd. A portion of the aseptic curd was placed in a sterile container and incubated for 48 h at either 30°C (for *L. lactis*) or 37°C (for *L. bulgaricus* and *S. thermophilus*). The pH of the curd was determined at 24 and 48 h.

Statistical Analysis

Processing trials were scheduled and analyzed using a split-plot experimental design. Three CO_2 treatment trials were run each processing day. Each trial contained milk with the same fat concentration and a different cheese starter culture, randomLy ordered (*L. bulgaricus*, *L. lactis*, or *S. thermophilus*). Triplicate processing days were scheduled for a total of 9 processing days and a total of 27 trials. Uninoculated milk controls were randomLy included as a fourth treatment trial for each fat level. General linear model (GLM) statistical analysis (SAS Institute, Inc., 1987), both whole plot and subplot analysis, was used to evaluate pH and microbiological responses.

RESULTS

Curd Development

Inoculated milk were processed in CO_2 under high pressure and produced curds after only 5 min. Processing trials for samples inoculated with *L. bulgaricus*, *L. lactis*, or *S. thermophilus* had average temperatures of 38.1, 38.1, or 38.6°C (standard deviation ≤ 1.6°C) and average pressures of 5.60, 5.56, or 5.58 MPa (standard deviation ≤ 0.12 MPa), respectively. This is within the target range suggested by Tomasula et al. (1995) for optimal curd production.

Curds were easily separated from whey. Only a small amount of casein fines were visible in the whey, but fat losses in the whey were noticeable for the 3.25% fat samples. Based on qualitative appearance and feel, non-fat curds were similar to the curd prepared by Tomasula *et. al.* (19) containing 80% moisture. Curds appeared drier as the fat content decreased. Curds containing *L. bulgaricus* were the firmest, and curds containing *L. lactis* were the softest. By the end of the second day of accelerated aging, curds had developed distinct cheese odors characteristic of the starter culture they contained.

Microbiological

Means for the number of viable starter cultures in milk containing 0, 1, or 3.25% fat, before nd after processing are shown in Fig. 1. Statistical analysis showed significant influence rom the type of starter culture but not from the level of fat in the milk. Milk (all fat levels ombined) inoculated with *L. bulgaricus*, *L. lactis*, or *S. thermophilus* contained 6.81, 7.35, r 6.75 log CFU/mL, respectively, before processing. After processing, the curd contained .68, 7.32, or 6.55 CFU/g, and the whey contained 5.05, 6.43, or 6.14 log CFU/mL, spectively. There was no significant difference between the cocci counts in the oculated milk and those found in the curd, while the lactobacillus showed approximately e log decrease in the curd. Control samples were overgrown with psychrotrophic bacteria d could not be counted.

anges in pH

ans for pH of milk, whey, and curds (fat levels combined for each culture) are shown Fig. 2. Processing in CO_2 resulted in both curd and whey having lower pH value than

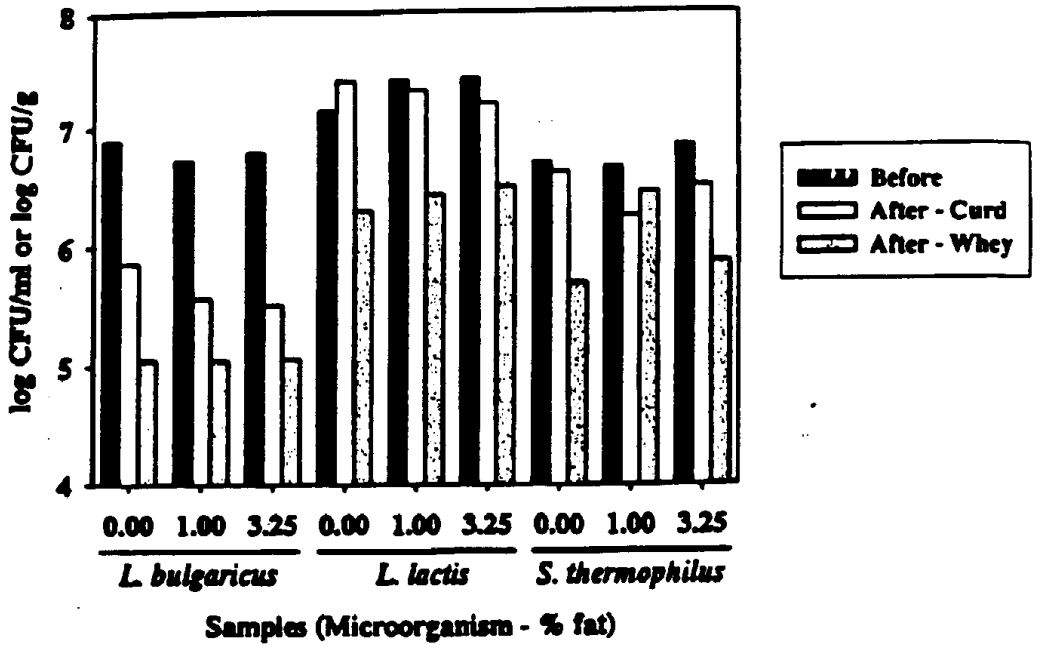

Figure 1. Means of colony forming units (CFU/mL or CFU/g) in milk containing 0, 1, or 3.25% fat and inoculated with either *L. bulgaricus*, *L. lactis* , or *S. thermophilus*, before and after CO_2 processing.

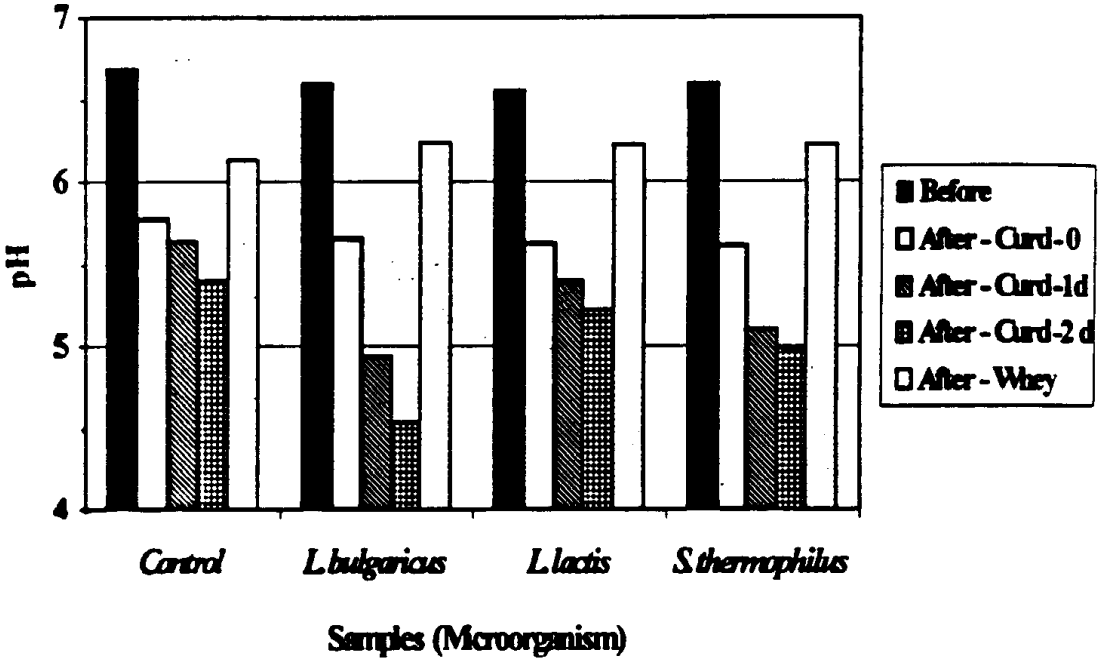

Figure 2. Means of pH of milk (average of all fat levels) inoculated with *L. bulgaricus*, *L. lactis*, or *S. thermophilus*, before and after CO_2 processing.

milk. After processing, the pH for the controls (uninoculated milk) decreased 0.90 units in the curd and 0.66 in the whey. The pH in the inoculated samples decreased between 0.93 and 0.99 in the curd and 0.32 to 0.37 in the whey. After 2 d of incubation, the curd containing *L. bulgaricus* showed the largest decrease in pH (1.12 units), while *L. lactis* decreased 0.62 and *S. thermophilus* decreased 0.40 units. The pH for control curds also decreased over the two days but only by 0.37 units.

DISCUSSION

Under high pressure, CO_2 dissolved into the aqueous phase of the milk and formed H^+ and HCO_3^-. This effectively lowered the pH of the milk near the isoelectric point of casein and precipitated the protein. When the pressure was released, CO_2 was reformed and easily vented from the chamber, and the precipitated casein curd and whey remained as separate fractions.

Gevaudan et al. (1996) studied the effect of CO_2 pressure up to 1.50 MPa on the colloidal calcium phosphate phase of milk. At this pressure, the milk remained fluid and was reduced to a pH of 4.9. They reported that inorganic colloidal calcium phosphate was irreversibly altered to other forms of calcium phosphate, although no change was noted in the distribution of protein to salts or in the concentration of inorganic phosphorus, calcium, or magnesium. Both Tomasula et al. (1995) and Strange et al. (1998) reported higher calcium concentrations in CO_2-prepared curds compared with commercially prepared caseinates. Strange *et al.* (1998) found insoluble aggregates in the casein which may have been complexes containing altered forms of calcium phosphate.

The 5 min CO_2 processing at 5.52 MPa and 38°C did not appear to have any detrimental effects on the starter cultures. Although considered high pressure, the 5.52 MPa used in this study was well below the 100 MPa required to disrupt hydrophobic interactions, biochemical reactions, and membrane integrity within microorganisms (Hoover et al., 1981). The processing temperature of 38°C used in this study was above the typical 30-35°C used for ripening and setting of cheese milk but within tolerant limits of most cheese starter cultures.

This study used a 1-L capacity batch chamber which produced quality curd but in limited amounts. Tomasula (1995) has developed a continuous process that would produce curds in a quantity suitable for commercial cheese production.

The CO_2 processing had minimal effect on the facultative anaerobic lactic acid starter cultures used in this study. The differences between the count of viable microbes in the milk and in the curd were not significant for the cocci strains and only a 1 log decrease in CFU/g for the lactobacillus strain. Carbonation of milk (up to 30 mM CO_2) has been shown to decrease the psychrotroph counts in raw milk (Amigo et al., 1995; King and Mabbitt, 1982; McCarney et al., 1995; Raus-Madicdo et al., 1996; Roberts and Torrey, 1988). When used in cheesemaking, carbonated milk resulted in cheese with lower numbers of psychrotrophs (Calvo et al., 1993; McCarney et al., 1995) and decreased proteolysis and lipolysis during aging (McCarney et al., 1995; Montilla et al., 1995; Uceda et al., 1994). In this study, the curd contained 5.7 to 7.3 log CFU/g. In traditional cheese manufacturing, the fresh curd should have about 9.0 log CFU/g to ensure adequate flavor and texture development by enzymes of the microorganisms (Stee;e amd Im;i. 1992). The

magnitude of the reduction in CFU due to CO_2 processing provides the basis for future research to determine ideal inoculation levels.

The CO_2 treatment did not hinder the ability of the starter cultures to produce lactic acid, as the pH of the curd continued to decrease after processing. In studies using carbonated milk to make cheese, the amount of lactic acid produced by starter cultures was lower, but the total lactic acid content was not significantly different from that of untreated samples (Calvo et al., 1993; Montilla et al., 1995). Cheeses were also found to have higher final pH (Calvo et al., 1993), and higher moisture content (McCarney et al., 1995) and required less rennet for coagulation (McCarney et al., 1995; Montilla et al., 1995).

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